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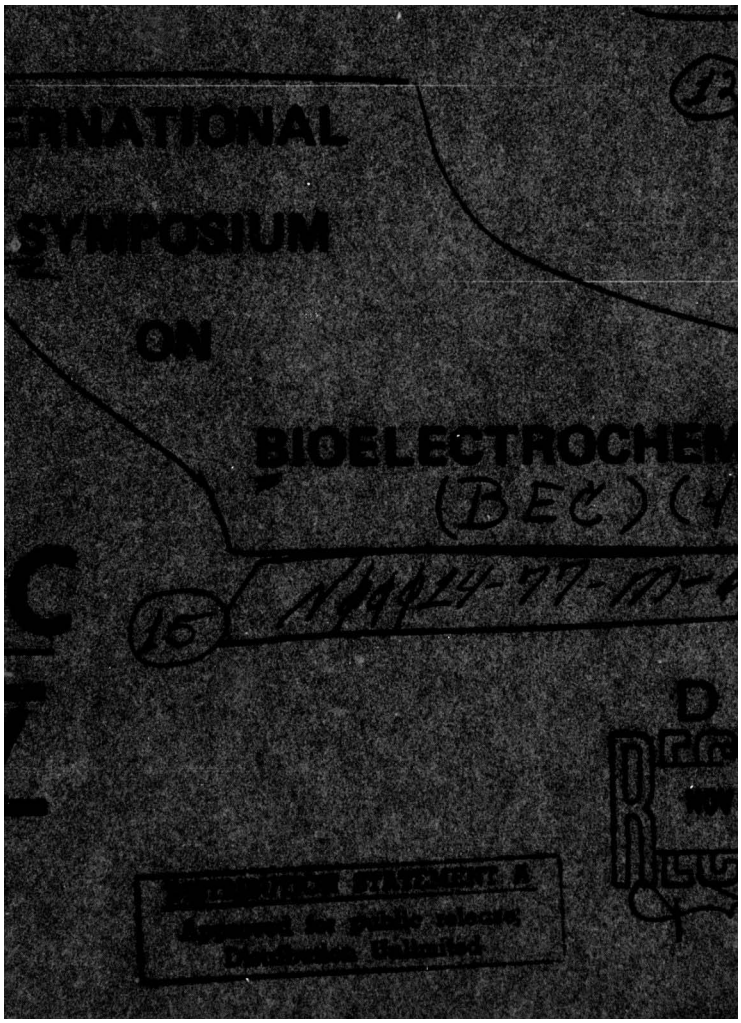
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ABSTRACTS

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STRUCTURAL TRANSITIONS INDUCED BY ELECTRIC FIELDS IN BIOPOLYMERS

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Abstract: Electric field effects play an important role in a number of vital cell processes. - After a short discussion on the localization of electric fields in biological structures a digression is given on the primary effects of electric field forces on molecules and molecular structures. The account on electrically induced chemical transformation includes (permanent and induced) dipole equilibria and ionic systems (field dissociation effects); problems of polyelectrolyte structures and reactions in membrane phases are also touched. The general analysis of electric field effects in biopolymer structures is extended to quantitative estimates of equilibrium shifts for given dipole moments, charge densities and field intensities. The amplifying effect of cooperativity in structural transitions, the occurrence of thermodynamically metastable states, hysteresis and threshold effects are outlined in the context of an electrical control of biochemical reactivity in general and memory recording in particular. - As an example of electrical chemical coupling in biopolymer structures, multi-stranded polynucleotide complexes and ribosomal rRNA are discussed. In these macromolecular systems changes in orientation relative to the field direction and conformational transformations (helix-coil transitions) result from the action of electric impulses similar in intensity and duration to nerve impulses.

ELECTROCHEMICAL STUDIES OF IMMOBILIZED PROTEINS

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Abstract: We have recently demonstrated (Anal. Chem., 48, 1679 (1976)) the feasibility of attaching an enzyme to an electrode surface for the purpose of demonstrating its catalytic behavior. As in the case of electrochemical kinetics, the overall reaction rate of biological reactions can be limited by substrate supply to the catalytic surface. Using a rotating ring disk electrode, the relative effects of enzyme kinetics and mass transport limited supply of substrate can be resolved. In addition, it is possible to study the effects of the enzyme microenvironment as it is influenced by conditions in the vicinity of the electrode surface. Applications of this technique to the study of electron transfer reactions at immobilized biosurfaces will be discussed from the point of view of fundamental studies as well as application to monitoring of biological fluids.

GENERAL CONTOURS OF BIOELECTROCHEMICAL BEHAVIOR OF NUCLEIC ACIDS AT CHARGED INTERFACES AS REVEALED BY MODEL STUDIES AT THE INTERFACE ELECTRODE/SOLUTION

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Abstract: The status of the knowledge assembled by advanced polarographic methods on the essential contours of the bioelectrochemical behavior of nucleic acids at charged interfaces as a consequence of their physicochemical parameters will be critically reviewed. The presentation will be based on the extended studies and the essential findings in the author's institute as well as on recent contributions of other laboratories. As has been shown first by us and later confirmed by other authors and by substantial further independent evidence established recently by us in the acid and alkaline range for the adsorbed zones of native DNA, the double helical conformation opens under the constraint of the adsorption forces and of the interfacial electric field, and passes intermediate conformational stages to the single stranded form a priori present for adsorbed denatured DNA. A similar behavior is generally to be expected for RNA yet many details have still to be studied.

In a fundamental biophysical sense it is to be concluded that analogous interfacial interactions of native DNA with charged biological interfaces occur also in the living cell causing a similar pattern of interfacial deconformation, e.g. in the in vivo replication of DNA in the primary stages of cell mitosis or in interactions with enveloping proteins.

CONFORMATIONAL TRANSITIONS OF DNA AT THE ELECTRODE SURFACE

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Abstract: The kinetics of transitions of DNA at the interface mercury-electrolyte is studied by measuring changes in the capacitance of the electrical double layer. We found that the shape of loose coils of DNA in solution is flattened in the adsorption layer depending on potential.

At the hanging mercury drop electrode the in-phase- and out-of-phase components of admittance were registered at different a.c. frequencies. From these measurements temperature dependent rearrangements of the DNA molecules in the adsorption layer between the following transition states (S_1 to S_4 at certain potentials U_1 to U_3) were detected : $S_1(U_1) \xrightarrow{\text{fast}} S_2(U_2) \xrightarrow{\text{slow}} S_3(U_3) \xrightarrow{\text{slow}} S_4$. The final state S_4 without any distinct a.c. peaks may be a thin layer of kinked DNA with a compactness as in its crystal. By complex formation with the antibiotic peptide netropsin the consecutive reactions are influenced, because the double helix may be screened against the electrode field effects. Models and theoretical explanation will be given taking into account the counter ion distribution in the double layers.

The mechanism of compactisation described here offers new possibilities in conformational transitions of DNA and a model of condensation as it is known e.g. for phages.

VOLTAMMETRIC STUDIES ON THE BIOELECTROCHEMICAL BEHAVIOUR OF IRRADIATED NATIVE DNA

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Abstract: Irradiation by γ-rays cause defects and modifications in the conformation of native DNA and may thus have effects on the genetic code. The irradiation effects were reflected in the voltammetric responses obtained for native DNA absorbed at the charged model interface mercury electrode/electrolyte solution. The dependence of the voltammetric responses measured by several independent methods on the radiation dose, the changes in molecular weight observed by ultracentrifuging, and comparisons with sonicated native DNA revealed as consequences of the irradiation: formation of single and double strand breaks and release of oligonucleotide units from the polynucleotide chain. Due to the excellent sensitivity of the applied advanced voltammetric methods the measurements could be extended to the detection of rather small conformational changes in native DNA at low radiation doses, an aspect of great significance in the elucidation of radiation hazard limits for this important biopolymer.

INTERACTIONS OF NUCLEIC ACIDS WITH THE ELECTRICALLY CHARGED SURFACE

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Abstract: The double-helical structure of deoxyribonucleic acid (DNA) may be distorted due to the interaction of a DNA molecule with the mercury electrode surface (1,2). At neutral pH an extensive "surface denaturation" occurs in a narrow potential range around -1.2 V (1,3). It is suggested that this "surface denaturation" results from the strains connected with the repulsion of certain segments of the molecule (anchored on the electrode) from the negatively charged surface. Recently it has been found that at potentials around ECM another type of DNA structural changes take place. These changes include an opening of the double helix and an easier exposition of bases to their environment, but they are limited to only a very small part of a DNA molecule. It is supposed that these local structural changes may be induced by the intensive electric field acting in the vicinity of the electrode surface. The marked dependence of structural changes of double-helical polynucleotides on the electrode potential is in close connection with the scope and limitations of various methods of electrochemical analysis in the nucleic acid structure research (4,5).

1. E. Palecek, Collection Czech. Chem. Comm. 39 (1974) 3449.
2. P. Valenta and H.W. Nurnberg, Biophys. Struct. Mechanism 1 (1974) 17.
3. V. Brabec and E. Palecek, Biophys. Chem. 4 (1976) 79.
4. E. Palecek in W.E. Cohn (ed.) "Progress in Nucleic Acid Research and Molecular Biology", vol. 18, Academic Press, New York 1976, p. 151.
5. E. Palecek, V. Brabec, F. Jelen and Z. Pechan, J. Electroanal. Chem. in press.

ANOMALOUS ADSORPTION ISOTHERMS OF SOME NUCLEOSIDES

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Abstract: The value of differential capacity measured in the vicinity of ECM potential usually decreases with increasing concentration of surface - active molecules; the adsorption isotherm has a positive slope in each point. With inosine and 5-bromouridine an anomalous concentration dependence of the differential capacity was observed. The adsorption isotherms of these nucleosides possess the segment with a negative slope.

The range of concentrations of 5-bromouridine at which the anomalous dependence of differential capacity on concentration is observed is the widest at neutral pH. With inosine the anomalous dependence on concentration is observed not only at neutral but also at alkalic pH. If an anomalous concentration dependence occurs, the potential of the minimum of capacity curves is shifted at highest concentrations towards negative values. The temperature dependence of the capacity curves of inosine and 5-bromouridine is anomalous as well. Nucleosides with similar structure as inosine and 5-bromouridine (e.g. xanthosine, uridine, 6-azauridine) show no anomalies in their adsorption behaviour (1). The anomalous adsorption behaviour of inosine and 5-bromouridine is explained by the self-association of the nucleoside molecules in the solution.

(1) Vetterl, V., Kovarikova, E., Nucleic Acids Res., Spec. Publ. No. 1, 93-96 (1975).

DIELECTRIC PROPERTIES OF THE ACID MUCOPOLY-SACCHARIDES CHONDROITIN SULFATES A, B, C, AND D

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Abstract: Dielectric measurements on the Chondroitin Sulfates A, B, C, and D demonstrate that these polysaccharides have relaxation times in the range of 10^{-8} seconds. The dielectric increment versus concentration is linear for the Chondroitin Sulfates with Chondroitin Sulfate A and C having a marked change in slope at a concentration of 1gm./liter. The variation of dielectric increment and relaxation times at different concentrations checked poorly with a continuous charge model. The Oosawa discrete charge site model for counterion fluctuations fit the experimental dielectric data rather well above 1gm./liter. The ratios of dielectric increment and relaxation times at two different concentrations were relatively constant and independent of the type of sulfate which is consistent with a fluctuating counterion model. Furthermore the discrete charge model can account for the temperature dependence of the relaxation times ratio and its independence of pH.

ACKNOWLEDGEMENTS

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THEORY OF ELECTRICAL POTENTIALS FOR LIVING CELLS

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Abstract: A brief sketch of the history of the theories of the electrical potentials of living cells is presented ending with a description of the model of Hodgkin and Katz, which has become the dominant theory in recent years. Why this theory should remain so popular while paper after paper have presented experimental data contradicting it, remains a mystery.

This paper then presents in somewhat greater detail the theory of electrical potentials according to the Association-Induction Hypothesis. In this theory the gross intracellular ion concentrations and the macroscopic membrane permeability to ions have no causal relation to the potential. Instead the potential is determined by the density and nature of fixed negative sites (e.g. β- and γ- carboxyl groups) and the nature and concentrations of monovalent cations in the surrounding medium. Both the resting and action potentials represent surface adsorption potentials at two different metastable equilibrium states. The equations describing these potentials are as follows:

$$\Psi = \frac{RT}{F} \ln [f^-] - \frac{RT}{F} \ln \left\{ 1 + K_K [K^+]_{ex} + K_{Na} [Na^+]_{ex} \right\} - \frac{RT}{F} \ln \left\{ \frac{[Na^+]_{ex}}{q_{Na}^{rest} [Na^+]_{ex}} \cdot \frac{\gamma_{ex}^{Na}}{\gamma_{in}^{Na}} \right\}. \quad (1)$$

$[K^+]_{ex}$ and $[Na^+]_{ex}$ are the concentration of external K^+ and Na^+ respectively. K_K and K_{Na} are their respective adsorption constants on the surface anionic sites. q_{Na}^{rest} is the equilibrium distribution constant of Na^+ between that in the water within the resting cell surface and that in the external medium. γ_{ex}^{Na} is the activity coefficient of Na^+ in the external medium; γ_{ex}^{Na} is the activity coefficient of free Na^+ in the water of the cell surface layer.

At rest, $K_K >> K_{Na}$ and $\gamma_S^{Na}/\gamma_{ex}^{Na} = q_{Na}^{rest}$,

Therefore

$$\Psi = \frac{RT}{F} \ln [f^-] - \frac{RT}{F} \ln \left\{ 1 + K_K [K^+]_{ex} \right\} . \quad (2)$$

The potential functions as a K^+ electrode.

After activation, $K_{Na} \rightarrow K_K$. As a result, $K_{Na}[Na^+]_{ex} >> K_K [K^+]$. Furthermore, concomitant depolarization of cell surface water leads to equilization of the activity coefficient of Na^+ in the cell water and external water, $\gamma_S^{Na} = \gamma_{ex}^{Na}$

Equation (1) now becomes:

$$\Psi = \frac{RT}{F} \ln [f^-] - \frac{RT}{F} \ln \left\{ 1 + K_{Na} [Na^+] \right\} - \frac{RT}{F} \ln \left\{ \frac{[Na^+]_{ex}}{q_{Na}^{rest} [Na^+]_{ex}} \right\} . \quad (3)$$

The cell surface then functions as a Na^+ electrode. The last term of Equation (3) also provides the basis for a $[Na^+]_{ex}$ dependent reversal of polarity or "overshoot".

Several sets of new experimental observations are discussed which have bearings on the validity of the theories of cellular electrical potentials.

ELECTRICAL POTENTIALS INDUCED BY CO_2 GRADIENTS IN PROTEIN SOLUTIONS

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Abstract: The diffusion of carbon dioxide in protein solutions causes, through chemical reactions, simultaneous fluxes of a number of ionic species. Bicarbonate and charged proteins are the most significant at or near physiological pH. Due to the great disparity between the mobilities of the simple anion and the macromolecule, a diffusion potential is established. The significance of such potentials with respect to total CO_2 transport in protein solutions has not been recognized. Experimental results demonstrate diffusion potentials induced by CO_2 gradients in hemoglobin solutions. The diffusion potentials are of such a magnitude that it impedes the facilitated transport due to the bicarbonate flux, and influences the distribution and the flux of all pertinent species in the solution.

LIPID INTERACTIONS WITH PROTEINS AND POLYPEPTIDES

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Abstract: Biological membranes are composite interaction products of lipids and proteins, where the proteins may either float freely within the lipid bilayer or at the lipid/water interface, or the membrane proteins may form networks by mutual interaction. The objective of any physicochemical investigation of the protein-lipid interaction is to determine the laws of nature which govern the structure of the biological membranes and relate them to their different functions.

The concentration of single, long-chain phospholipid molecules is negligible in aqueous solution, and proteins in this domain can interact only with lipid aggregates which assume layered structures of different shapes, e.g. monolayers, planar bilayers, bilayer vesicles, and multishell liposomes. Only the monolayer and bilayer structures will be considered in the forthcoming discussion.

The adsorption and the penetration of a series of synthetic and natural polypeptides and proteins, onto and into different lipid monolayers at their equilibrium spreading pressure, were investigated. The adsorption was determined by measuring the surface radioactivity of the ^{14}C or ^{3}H labelled polypeptides. The degree of penetration was derived from the effect on the capacitance of the lipid layer and on its permeability to different substances undergoing an electrode process as measured on a mercury dropping electrode.

Specific penetration of different domains with attached electroactive groups on the penetrating molecule could be obtained directly from the electrode response across the lipid layer. Correlation was found between penetration of lipid monolayers, effect on conductance of lipid bilayers and conformational changes and phase transitions in the interaction products between lipid bilayer vesicles and the respective polypeptides or proteins. The latter was investigated by differential scanning calorimetry, circular dichroism and fluorescence. The results were found to be in agreement with those obtained in other laboratories by different methods. Particular attention was given to the electrical field effects on the adsorption and penetration.

ORGANIC ELECTROSORPTION AS A PROBE OF WATER STRUCTURE IN THE INNER LAYER

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Abstract: A theory of noncongruent electrosorption of organic compounds at the metal solution interface is proposed in which the inner (compact) part of the electrical double layer is treated as a two-component nonelectrolyte solution called the surface solution. By means of this theory it is possible to calculate from experimental electrosorption data the excess electrochemical Gibbs free energy of mixing of the surface solution as well as the activities and activity coefficients of the adsorbed organic compound and of the adsorbed water in the inner layer. It is shown that in a certain range of the excess charge density on the metal the dilute surface solutions exhibit positive deviations from Raoult's law, and it is proposed that, like bulk aqueous solutions of organic compounds, the thermodynamic properties are under entropy control. On this basis it is concluded that the so-called modified Flory-Huggins electrosorption isotherm is probably incorrect because the statistical mechanical theory on which that isotherm is based predicts that the excess electrochemical entropy of mixing of the surface solution will be positive. A new parameter which results from this theory, the standard electrochemical fugacity ratio of the adsorbed organic compound, is shown to be an indicator of the water structure in the inner layer. It is proposed that, depending on the magnitude of the electric field in the inner layer, the water in the inner layer retains a remnant of its bulk-like structure. Application of this theory to experimental electrosorption data will be illustrated. Possible applications of this theory to the problem of water structure at biological interfaces will be discussed.

BINDING OF CATIONS TO PHOSPHATIDYLSERINE VESICLES

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Abstract: The binding of Ca^{2+} and Mg^{2+} to phosphatidylserine (PS) vesicles is studied experimentally and analysed. The surface potential and charge density are found for any given concentration of both monovalent and divalent cations in solution from a modified Gouy Chapman equation. An analytical expression is given for the amount of cations concentrated in the double layer region, which is treated distinctly from the amount of tightly bound cations.

The explanation of the binding results of Ca and Mg to PS required taking into account the binding of Na to PS. The computed binding of Na to PS is in accord with recent results of NMR and vesicle aggregation studies. The calculated surface potentials of PS in the presence of Ca and Na are in a reasonable agreement with the previously measured zeta potentials.

Our results indicate that Ca^{2+} has a ten-fold greater intrinsic binding constant than Mg^{2+} for PS vesicles.

DEFORMATIONAL INSTABILITY OF BIOMEMBRANES INDUCED BY CHEMICAL REACTIONS AND ELECTRICAL INTERACTIONS

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Abstract: This theoretical study shows that one of the important functions of biological cells, i.e. the deformation of the cell membrane, may be initiated by electrochemical phenomena.

The membrane separating the external and the internal electrolytic solutions, is considered as an interface between two immiscible fluids (I). A linear normal mode analysis of the deformational instability of this membrane is done. The model used is characterized by the presence of ion and dipole layers and by the chemical adsorption-desorption of charged molecules from the bulk solutions. It is applicable either to artificial lipid membranes, either to biological membranes, composed by lipids and proteins. The present analysis, based on membrane structural properties may be relevant to explain some specific cell membrane functions, as phagocytosis or pseudopode formation.

For highly concentrated solutions, the effects induced by diffuse layers may be neglected and the stability depends on change in interactions between individual charges and dipoles when the interface is perturbed from its original flat shape. The convective motion of the bulk fluids obeys Navier-Stokes equation. Boundary conditions at the membrane are obtained from conservation laws of momentum, mass and charge. The membrane is considered as a bidimensional visco-elastic fluid. The fluctuation of surface tension is coupled to the fluctuation of the concentrations of the chemical species by a state equation, supposing local equilibrium. The secular equation shows first that the electrical terms have destabilizing effects, opposed to pure mechanical surface tension effects. Secondly, the system is also unstable when the chemical adsorption occurs far from equilibrium (2), and overcomes stabilizing effects such as the viscosities of the bulk phase and of the membrane, and the diffusion of surface molecules (phospholipids or proteins).

(I) A. Sanfeld and A. Steinchen, Biophysical Chemistry 3, 99-106, (1975).
(2) G. Nicolis, I. Prigogine, Self organisation in non-equilibrium systems, Wiley Inter sciences, New York (1977).

[+]Supported by the Belgian Government : Actions Concertees, Convention no 76/81-II 3.
[*]Supported by a grant from C.N.Pq (Brazil).

DIRECT CONVERSION OF CHEMICAL ENERGY INTO MECHANICAL ENERGY

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Abstract: Spontaneous motions can occur at an oil-water interface when the phases which contain ionized compounds - one of them is surface active - are far from partition equilibrium. They appear differently according to the experimental conditions: either both solutions come into contact with a glass wall, then a motion occurs at the interface as a wave, deforming the meniscus, and stirring the whole interface - or both solutions are simply brought into contact and local contractions or expansions of the interface plane can be observed. Moreover the movements of a drop of the oil phase running within the aqueous phase along a glass wall look like these of some elementary living organisms.

In a first set of experiments these motions were obtained from a system containing KI_3 in the organic phase and octadecyltrimethylammonium chloride in the aqueous phase. We pointed out the transfer of I_3^- from the organic to the aqueous phase which can be considered as an electrochemical reaction as far as ions could be considered as charge carriers. But an oxydoreduction reaction is not necessarily required to obtain the movement. Indeed we realized that an acido-basic reaction between picric acid in nitrobenzene and octadecyltrimethylammonium hydroxyde in the water is sufficient to induce the movements. In each case we observed correlations between the variations of the pH near the interface, the potential difference between the two phases and the interfacial tension. The variation of these parameters during the time could be related to a chemical interfacial oscillating reaction.

MEASUREMENT OF INTERMOLECULAR FORCES BETWEEN AND WITHIN PHOSPHOLIPID MEMBRANES

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Abstract:

We have used three complementary methods to measure the work required to remove water from a multilayer stack of phospholipid bilayer membranes. These methods are (1) to expose the multilayer to a dextran solution which exerts an osmotic pressure on it, (2) to place the multilayer in a pressure cell and squeeze out the water through a semipermeable membrane, (3) to expose the multilayer to water vapors of controlled pressures. Withdrawal of water brings the bilayer membranes closer together as well as causes molecules in the same bilayer to pack closer together. These changes are accurately detected by x-ray diffraction. We convert the work of removal of water into the distinct works of pushing membranes together and of disturbing the bilayer from its equilibrium packing.

Lecithin bilayers repel with an exponentially decaying force (decay distance $\sim$2Å) that reaches several thousand atmospheres as the membranes near contact. This "hydration" force is seen also between charged phospholipids and in those cases it overshadows electrostatic double layer forces for separations less than 20Å. For this reason we suggest that one must take account of hydration forces in analysing processes such as vesicle-membrane contact and fusion.

MOLECULAR INTERACTIONS OF SPECTRIN CAUSED BY CA^{2+}-BINDING

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Abstract: Spectrin is known to be a protein with a molecular mass of 460,000 daltons - situated at the inner surface of the erythrocyte membrane. More than 1500 side groups of polar amino acids may constitute either bond or dissociation balances with the solvent and with added ligands.

The dissociation of H^+ was measured with pH-microrod electrodes. The different amino acids may be identified by determining the dissociation enthalpies. The ΔH values found by our experiments differed widely from the expected ones. These differences suggest effects as changes of conformation or aggregation during titrating.

Checking the velocities of sedimentation with an ultracentrifuge resulted in a faster moving of the protein with increasing concentration of the salt besides the expected dependence of the pH-value. The reason for this behavior may be either electrostatic effects between the central ion and the surrounding cloud of opposite sign, or it can be considered as a hint for a change of conformation of the protein.

The second assumption is at the first sight less probable as the structure of proteins is mostly fixed. The free energy of the system will reach a minimum by inserting the unpolar side chains of the amino acids in the interior of the molecules, so that no water can diminish the entropy-term of the protein hydrates by condensation. With such a large and highly charged molecule as spectrin the electrostatic repulsion will counteract to equally charged groups, so that the solvent is enabled to penetrate the protein by its unfolding.

Controlling this assumption is possible by determination of the electrostatic interaction of the Ca^{2+}-binding to spectrin. Using a flamephotometer and a Ca^{2+}-sensitive electrode two bonding branches were found in the Scatchard plot, both of which obeyed the laws of multiple equilibria without electrostatic interaction. That means that no electrostatic energy is absorbed by the repulsion of ligand-ions but by a change of conformation.

From these measurements therefore was concluded that with spectrin contrary to most of the investigated proteins the minimum of free energy of the solved form is preferably influenced by an electrostatic term which causes the structure of the protein to be varied. This interpretation is decisively supported by directly determining the Stokes-radius of spectrin with column chromatographic methods in dependance of the salt concentration.

INTERACTIONS IN MIXED MONOLAYERS OF MYELIN STRUCTURAL PROTEIN AND A NATURAL PHOSPHOLIPID

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Abstract: The apoprotein A of the Folch-Lees Proteolipid is a structural protein involved in the building of myelin in central nervous system (brain). It is positively charged at physiological pH and interacts strongly with acidic phospholipids. The mixed films of A with a typical acidic phospholipid L : the phosphatidyl inositol monophosphate have been studied as a possible model for the protein-lipid interaction in myelin. Surface pressure, surface potential and $^{45}Ca^{2+}$ binding have been measured for various ratios of A/L in the mixed films at room temperature and pH = 5.5. The interpretation is concerned only with the results obtained at the maximum surface density of the film one molecule thick or collapse. It is found that the effect of the film composition or ratio A/L on surface pressure and potential are relevant to the state - homogeneous or heterogeneous - of the mixed film. The analogy with the collapse of proteins in biological or model membranes is stressed.

INTERACTIONS BETWEEN RED CELL MEMBRANES

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Abstract: Red blood cells form aggregates in the presence of macromolecules which can bridge the membrane surfaces of adjacent cells. The aggregation-disaggregation phenomena are governed by an energy balance at the cell surfaces. The aggregating energy due to the binding of the bridging macromolecule to the membrane surface (E_B) is counteracted by several disaggregating energies, which include electrostatic repulsive energy (E_E), membrane resistive energy (E_m) and the work done by shear stress (E_S).

Red cell aggregation induced by neutral and charged polymers has been quantified while each of the above energies is systematically varied: E_B is changed by using different types of polymers over a wide range of concentration; E_E is altered by removing the red cell sialic acid with neuraminidase and by varying the ionic strength; E_m is assessed by theoretical modeling of membrane bending modulus; and E_S is varied by controlled shearing of the aggregates. The results generate quantitative information on energy balance in red cell aggregation and allow an estimation of the magnitude of the molecular energy involved in the binding of various types of macromolecules to the red cell membrane.
(Supported by U.S.P.H.S. Grant HL-16851).

THE RELEVANCE OF ELECTRIC FIELD INDUCED CHANGES IN THE MEMBRANE STRUCTURE TO BASIC MEMBRANE RESEARCH AND CLINICAL THERAPEUTICS AND DIAGNOSIS

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Abstract: Electric field pulses of short duration above a certain threshold value induce reversible changes in the membrane structure which result in an increased membrane permeability (dielectric breakdown). The electrical isolating properties of the cell membrane are restored in seconds to hours depending on the species used and the experimental procedure. A detailed theory of the underlying mechanism leading to dielectric breakdown is presented on the basis of the electro-mechanical forces and the polarization processes occurring in the membrane during field application. In addition, the connection between both the macroscopic field induced changes and the molecular processes in the membrane are revealed. Convincing experimental evidence is given that the membrane can be considerably compressed by high electric field strengths. The transient changes in the membrane permeability after field application can be used to allow the incorporation of radionuclides, macromolecules (DNA), genes and organelles into cells. Due to the resealing of the cell membrane, such foreign particles will therefore be trapped inside the cells. Red blood cells and lymphocytes loaded with enzymes and drugs can be used both for long-term applications in human therapeutics, and for short-term, organ-specific administration of drugs. Organ-specificity of such loaded cells is achieved by incorporating ultrafine magnetic particles into the cells at the time of loading and guiding of the cells in the blood circulation by external magnetic field application.

Finally, the stimulating effect of the applied electric field in cell fusion is described.

LYMPHOCYTE-LYMPHOCYTE HISTOCOMPATIBILITY: A FUNCTION OF CELL SURFACE CHARGE CHARACTERISTICS

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Abstract: Success or failure of renal transplants, depends on cellular genetic compatibility of the donor and recipient. Techniques for evaluating compatibility include human lymphocyte antigen (HLA) and mixed lymphocyte culture typing (MLC). Analysis of the predictive value of these tests demonstrates that they are inaccurate in 50% of cadaveric organ transplants.

Using microelectrophoresis (ME), Sawyer et al. conducted a study of 40 renal transplant pairs. The electrophoretic mobility (EM) of lymphocytes from the donor and recipient were measured independently and following a 30 minute incubation at 30°C. Similar studies were conducted on histocompatible and incompatible rat and mouse lymphocytes. Sixty animal experiments were completed. The results of this combined 2 year study are as follows; (1) significant change in the EM of incompatible lymphocytes (mixed lymphocyte electrophoretic mobility MLEM) can be measured by (ME), (2) when used to predict renal transplantation success rates this test had a predictive value of 75%, (3) compatible lymphocytes from animal strains possess similar EM's, (4) incompatible cells possess similar EM's, (5) the incompatible MLEM is significantly different from the EM of the control.

Based on these findings the following research was designed to evaluate the role of the cell surface charge and/or chemistry in regulating or influencing lymphocyte-lymphocyte interactions. Using leukopack isolation techniques, experiments were conducted on pure rat 'T' and 'B' cell populations. Fifty units of neuraminidase and 0.2mg of anti-immunogamma globulin (IgG)/ 1 ml of cell suspension were evaluated. Incompatible cells in the presence of the above agents were incubated for 30 minutes and 90 minutes respectively and the MLEM recorded. This preliminary study indicates the following; (1) 'T' cell populations possess a decreased MLEM (14%) in the presence of neuraminidase, (2) the effect on 'B' cells is less (10%), (3) anti-IgG had no effect on the MLEM but did decrease the EM of the independent samples.

The results imply the following; (1) neuraminidase cleaves a greater number of sialic acid residues from 'T' cells than 'B' cells, (2) neuraminidase treatment augments the MLEM by what appears to be a synergistic action on the independent samples. Neuraminidase may effect the electrostatic repulsive forces between the charged lymphocytes, allowing greater cell-cell contact. Neuraminidase may uncover specific receptor sites by surface conformational changes, (3) anti-IgG is complexing the surface active IgG components of 'B' cells thus reducing cell-cell interaction.

In summary, cell electrophoresis appears to be a rapid, accurate method of evaluating histocompatibility for renal transplantation prior to chemotherapy as well as evaluating lymphocyte-lymphocyte interaction as a function of cell surface charge characteristics. This research indicates the importance of bioelectrochemistry in understanding membrane-membrane processes.

FLUORESCENT DYES, MEMBRANE POTENTIALS AND TRANSPORT PROPERTIES OF RED BLOOD CELLS

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Fluorescent dyes such as DiS-$C_3(5)$, (3,3'-dipropyl-thiadicarbocyanine iodide), can be used to monitor membrane potentials (V_m) of cells in situations where determination of V_m by means of microelectrodes have not been possible, e.g. mammalian red blood cells. Changes in the fluorescence intensity of DiS-$C_3(5)$ can be quantitatively related to V_m even though dye calibration with V_m might vary with different experimental circumstances. In addition the dye can be used to follow transient changes in V_m. Analysis of the effects of a variety of agents together with alterations in the ionic conditions on V_m, has provided a way of studying the permeability characteristics (ionic conductances) of the human red cell membrane to Na, K, Cl and protons.

THE SURFACE COMPARTMENT MODEL OF THE NATURAL MEMBRANE

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Abstract: In order to characterize many physiological phenomena it would be desirable to develop a continuous treatment of ion flow through a membrane, i.e., to describe ion concentrations and electrical potential as functions of a space coordinate across an entire system. However, it is possible to approximate the description with the aid of the Surface Compartment Model (SCM) described earlier (1). The SCM divides the entire system into discrete regions: two bulk reservoirs (the inner and outer solutions), two surface layers (on the inner and outer faces of the membrane) and the ultra-thin membrane itself. The surface layers are regions where the ionic concentrations and the electrical potentials vary significantly with the space coordinates, but average values are chosen for these quantities and they are assumed to be uniform throughout the region. Ion flux and conservation (of mass and charge) equations can be written in terms of the various compartments assuming that the transport processes are discontinuous across the interfaces between compartments and that the fluxes are proportional to the differences in the electro-chemical potential (i.e. Nernst-Planck type expressions).

Using the SCM it can be shown that the concentrations of ions in the surface regions of excitable membranes and the calculated permeabilities of the membranes to these ions are quite different from those that are generally accepted on the basis of bulk concentrations. Furthermore, it appears that small changes in the surface charge can alter the ionic concentrations at the membrane surfaces and therefore, the ionic fluxes. This is especially true if appreciable numbers of ions are bound to the membrane surfaces and ion exchange processes occur as a result of current flow. The physiological mechanisms of excitation and active transport, processes that involve unusual ion fluxes in terms of bulk concentrations and potentials, are not yet understood. The SCM approach suggests the possibility that the unusual ion fluxes may actually be occurring in the expected directions during transient states brought about by electrical currents or chemical reactions.
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(1) M. Blank - J. Colloid Science 20:933, 1965.

THE CELL-TO-CELL MEMBRANE CHANNEL AND ITS PERMEABILITY REGULATION

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Abstract: A wide variety of cells make junctions containing membrane channels that connect the cell interiors with each other. These channels are permeable to molecules up to about 1400 daltons and are well insulated from the exterior. The estimated single-channel electrical conductance is 10^{-10} mho (lower limit). The channel permeability is regulated by Ca^{2+}. As the cytoplasmic concentration in the junctional domain is raised from its normal level of $< 10^{-7}$ to $> 5 \times 10^{-5}$ M, channel permeability diminishes selectively; the molecular size limit for permeation, probed with a graded series of fluorescent peptide probes, diminishes gradually; at concentrations $> 5 \times 10^{-5}$ M the channels are closed even to the smallest inorganic ions. The graded control by Ca^{2} offers, in principle, a powerful means for selective transmission of intercellular molecular signals.

THE INFLUENCE OF THE COMPOSITION OF THE BATHING SOLUTIONS ON FROGSKIN MEMBRANE POTENTIAL

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Abstract: The extreme dependence of the over-all electrical potential of frogskin membrane on the composition of the solutions with which it is in contact inside and outside is studied. Effects observed include: High ionic strength in Na, K or Ca both sides depresses, the effect being greater on the outside; lower ionic strength in Na makes little difference except at very low concentration while K and Ca make little difference even at zero concentration. With different solutions on the inside vs. the outside very low Na hurt the potential greatly from the inside but not so from the outside. Thus, application of Na thermodynamic potentials from inside to outside and reversely had little bearing on the membrane potential and on Na transport (not measurable tracerwise) between the solutions through the membrane in either direction. Low ionic strength as a whole on the inside lowers the potential. The outside is much less sensitive thus. H^+ is released by both sides with time, moreso from the outside. The membrane potential was stable to pH = 5 and decreased below this value the outside being more sensitive than the inside. In high ionic strength or in low strength (distilled water) the sign of the potential is reversed, high Ca being more effective than high Na; the inside is more responsive to the distilled water than the outside. Thus isoelectric ionic concentrations are indicated. The overall potential seems to be the algebraic addition of an "inside potential" and an "outside one". The effects of various polyvalent cations were observed to fall into three general categories. Class I (Mg^{+2}, Sr^{+2}, Ba^{+2}) showed little difference. In Class II there was an immediate increase in potential followed by decrease (Mn^{+2}, Co^{+2}, Ni^{+2}, Cu^{+2}, Zn^{+2}, Cd^{+2}, Pb^{+2}, Fe^{+2}, Ce^{+3}, La^{+3}, Cr^{+3}, Al^{+3}, Sc^{+3}, Y^{+3}, Th^{+4}) when application was on the outside. There was little difference from the inside. In Class III (Be^{+2}, Hg^{+2}, UO_2^{+2}) the effect was to poison, the membrane being affected more readily from the outside than the inside. Recovery upon reexposure to Ringer's was studied in general. As an example of extremes there was no recovery from the Hg but Be showed higher potential than originally.

INTERFACIAL ELECTROCHEMICAL PHENOMENA CONTROL ION TRANSPORT IN THE ISOLATED TOAD BLADDER MEMBRANE SYSTEM

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Abstract: The basic mechanism involved in the passive transport of Na^+ in the epithelial cell system of the toad bladder has been investigated using a new electrochemical technique designed to have sufficient time resolution to separate potential dependent interfacial steps from those involving membrane and aqueous transport. Basically the approach utilizes Laplace plane analysis by which the system function (impedance) could be examined from 1Hz to 1MHz. The results thus obtained are compared to several membrane impedance models which take into account fast interfacial steps (specific adsorption, etc.) coupled with transport. This analysis appears to unmask a fast adsorption process coupled with the previously isolated Na^+ transport pathway. The specific adsorption step may involve Ca^{2+} and sheds light upon earlier results wherein it was found that Na^+ penetration (partitioning) is by far the rate limiting step in its membrane transport.

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EXPERIMENTAL AND INTERPRETIVE PROBLEMS ASSOCIATED WITH MULTICOMPONENT REDOX ENZYMES

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Abstract: For the past several years, our research efforts have been directed to the unravelling of the energetics and mechanism of electron-transfer to and from cytochrome c oxidase, the "premier" copper-heme protein of the mammalian respiratory system. During the course of this work, we have sought to develop appropriate experimental methods to electrochemically couple such enzymes to the electrode for conveniently pumping the redox levels and optically monitoring the redox centers. Since the large peripheral structure of the protein governs and "inhibits" facile heterogeneous transfer with an electrode, much of our work has dealt with the indirect coulometric titrations of these components. From the comparison of the experimental and computer simulated optical absorbance versus electrochemical charge curves, evaluation of the energetics of both the visible and invisible components have been possible. More recent work has been directed toward application of these methods to probe the behavior of intact submitochondrial particles. Some of the experimental approaches and problems associated with particle studies will be discussed, including the design and fabrication of "tailor-made" electrode surfaces for coupling to bio-components.

Energetics coupled with kinetic information (from our laboratory and others) will be reviewed and the current ideas about the mechanistic behavior of cytochrome c oxidase will be presented.

ELECTRON TRANSFER PATHS IN THE PROSTHETIC GROUPS OF HEMOPROTEINS

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Abstract: The ferro-ferricytochrome c couple is the structurally best characterized link of the terminal oxidation chain of cellular respiration: its cross-reaction rates with a variety of low molecular weight redox couples (e.g., iron EDTA, cobalt orthophenanthroline, etc.) and relevant isotopic electron self-exchange rates have been recently reported to correlate in accordance with the Marcus Theory. That Theory also predicts similar correlations with corresponding electrode kinetics and electrochemical mechanisms. Accordingly, we have investigated the electrochemical behavior of various fragments of cytochrome c identified in Table I.

Table I. Model Compounds Related to the Prosthetic Group of Cytochrome c

Moiety	Chemical Description	Method of Preparation
porphyrin c	2,4 bis-cysteine adduct of protoporphyrin IX	synthesis
heme c	iron complex of porphyrin c	synthesis
HP8 (octapeptide)	heme c plus amino-acid residues 14 through 21	enzymatic degradation
HP9 (nonapeptide)	heme c plus amino-acid residues 14 through 22	enzymatic degradation
HP11 (undecapeptide)	heme c plus amino-acid residues 11 through 21	enzymatic degradation

The peptide fragments HP8, HP9 and HP11 exhibited qualitatively the fundamental characteristic of heme c, viz., their sole electron transfer reactivity was associated with the ferric-ferrous redox process. The relevant standard potentials and electrochemical rate constants varied as follows:

	k^o / cm/sec	E^o / Volt vs. SCE
heme c	$(2\text{-}5) \times 10^{-4}$	-0.300
HP11	$(2\text{-}6) \times 10^{-3}$	-0.265
HP9	$(1.5\text{-}4) \times 10^{-3}$	-0.270
HP8	$(1.5\text{-}3.5) \times 10^{-3}$	-0.270

The significance of these experimental findings will be assessed critically in the context of extensive recent results (reported by several investigators) on electrochemical effects engendered by axial ligands in hemochromes, and by electrophilic and nucleophilic substituents on the periphery of the equatorial ligand of metalloporphyrins. The crucial question will be discussed, whether the ferrous-ferric electron transfer in-and-out of the prosthetic group of hemoproteins implicates the equatorial porphyrin moiety, or the axial coordination orbitals, or both.

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EFFECT OF BRØNSTED AND LEWIS ACIDS ON THE ELECTROCHEMICAL REDUCTION OF NAD^+

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Abstract: Electrochemically, NAD^+ (nicotinamide adenine dinucleotide; DPN^+; coenzyme I) is reduced in two steps: a pH-independent one-electron reduction to the free radical, which, at more negative potential, is reduced in a one-electron, one-proton proton process to NADH. The effects on the electrochemical pattern of complexation with Lewis acids known to be involved biologically with NAD^+ and of Brønsted acids (H^+; H_2O; NH_4^+) have been considered. The second electron-transfer step seems to involve preprotonation of the free radical; in the pH region, where this step is electrochemically observable, water may be the protonating agent. Addition of ammonium ion at constant pH facilitates the second reduction, but has no effect on the first step. Potentiometric titration indicates that both Ca(II) and Zn(II) form weak 1:1 complexes with NAD^+ at pH 7.2; d.c. and a.c. polarography support such complexation. The nature and effects of these complexes have been explored, e.g., Ca(II) at pH 9.6 has only a slight effect on the first reduction step but a marked effect on the second.

RATES OF ELECTRON TRANSFER OF METALLOPORPHYRIN

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Abstract: The heterogenous rates of electron transfer of porphyrins and metalloporphyrins. These rates are influenced by a variety of factors the study of which may lead to an understanding of mitochondrial electron transfer processes. Important factors which influence k^{o}'s including ligand binding strength, ligand exchange or spin state coupled to reduction, porphyrin substituents, and solvent and other molecular environmental factors. Data will be presented on all of the above effects. Perhaps the most interesting but poorly understood effects are those of solvent. The use of mixed solvents give rise to complex effects, that cannot simply be explained by donor number of dielectric constant. It is the aim of this work to not only model cytochromes but also the environment of the electron transfer site and try to elucidate electron transfer mechanisms.

REDOX REACTIONS AND ANTITUMOR ACTIVITY OF TETRA-μ-CARBOXYLATODIRHODIUM(II)

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Abstract: It has been demonstrated that tetra-μ-carboxylatodirhodium(II) complexes are effective antitumor agents. Because of the speculation that antitumor activity may be related to redox reactions of these complexes, we have undertaken an investigation of both the oxidation and reduction of a series of Rhodium(II) carboxylates, $Rh_2(O_2CR)_4$, where R in the bridging acid group has been extensively varied. The redox reactions of these complexes will be discussed in terms of solvent interaction, nature of the substituent R, number of electrons transferred and known antitumor activity of the reactant. Results on antitumor activity of the oxidized and reduced products will also be presented.

THE USE OF CHEMICALLY MODIFIED ELECTRODES AS CLINICAL SENSORS

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Abstract: Chemical modifications of the surfaces of electrodes such as SnO_2, TiO_2, RuO, graphite and glassy carbon, have been widely developed by the formation of covalent binding with organic groups directly or via silyl ether moieties. Recently the chemically modified electrodes (CME) have been also applied to analytical sensors using common electrochemical methods.

In addition to the above sensors, new sensors were produced by chemical binding of hydroxy groups on the insulated gate film of "Liquid-Oxide-Semiconductor Field-Effect-Transistor(LOS-FET)" with organic ligands and these will be reported in this paper. The LOS -FET coated by polymers which suspend ligand groups has already been reported by other authors, but it is defective as the ligands are unstable and exhibits high time dependence. On the contrary, the modified LOS-FET is free from these defects and furthermore has the advantages of indifference to electrode resistance. It is small in size, easily mass produced, easily used, and has long lifetime, etc.

As an example of clinical sensors, the LOS-FET modified with crown ethers or cryptants was suitable as a sensor of potassium ion in solutions. The gate-source potential of the chemical modified LOS-FET followed the Nernst equation over a wide K^+ concentration range of 10^{-1} to 10^{-5} mole/l. Similarly modified LOS-FET with variously bound ligands can be expected to be useful clinical sensors.

SURFACE MODIFICATION OF $(SN)_x$ ELECTRODES

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Abstract: The highly conducting inorganic polymer, polymeric sulfur nitride, $(SN)_x$, is being employed as an electrode material. Due to its chemical and electrical anisotropy the electrode behavior of both crystal planes can be studied independently. As these surfaces can be chemically modified more easily than metal oxide or carbon electrodes, it should be possible to attach biological moieties, e.g., the active site of an enzyme, to the $(SN)_x$ surface and study faradaic processes with "substrate" materials. Enzyme mechanisms could be elucidated by employing electrochemical techniques. These systems could also serve as models to probe electron transfer processes or electrical interactions which occur at membranes.

In order to pursue these ends, $(SN)_x$ was first characterized as an electrode material by using linear sweep cyclic voltammetry and chronoamperometry with and without electroactive species present in a variety of supporting electrolytes. Redox systems whose behavior is well known on other electrode materials were chosen. Certain metal cations were found to interact very strongly with the $(SN)_x$ surface. These "derivitized" electrodes may yield unusual catalytic properties.

A HIGHLY SENSITIVE GLUCOSE ELECTRODE USING COLLAGEN ENZYMATIC FILMS

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Abstract: As industrial collagen films have shown excellent properties for enzyme linkage (1-3), we have studied the behavior of such enzymatic films associated with electrochemical detection.

We have realized a glucose sensor whose main characteristics are:

- a high selectivity even in the presence of hydrogen peroxide, ascorbate, tyrosine, uric acid...
- a very high sensitivity for glucose c.a. 0.5μM
- a short response time (30-45s)
- a very large concentration range for which the response is proportional to glucose concentration (5.10^{-7} to 2.10^{-3}M)
- a good stability both of the membrane activity and of the sensor response.

The influence of the film structure and porosity and of its enzymatic activity on the sensor response is discussed in terms of rate controlling step.

1. P. Coulet, J. Julliard and D. Gautheron Fr. Patent no 73-23-283 (ANVAR)
2. P. Coulet, These de Doctorat 3e cycle, Universite Claude Bernard, Villeurbanne (1973).
3. P. Coulet, J. Julliard and D. Gautheron. Biotechnology and Bioengineering 16 1055-1068 (1976).

THE MITOCHONDRIAL RESPIRATORY CHAIN: STOICHIOMETRY AND ORGANIZATION OF THE REDOX COMPONENTS IN THE ENERGY TRANSDUCTION SITES

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Abstract: Mitochondrial oxidative phosphorylation is the product of the coupling between the exergonic oxidation of reduced substrates by molecular oxygen and the endergonic phosphorylation of adenosine diphosphate (ADP) to adenosine triphosphate (ATP). The redox reactions occur in the respiratory chain, a system of redox components which constitute an integral part of the mitochondrial membrane. In order to identify and characterize the redox components of the respiratory chain, we have utilized a combination of methods including redox potentiometry, titration with chemical oxidants and reductants, and coulometry. In purified enzymatically active cytochrome c oxidase, there are 4 one-electron redox components in equal stoichiometry - cytochromes a and a_3 and two copper atoms - with two (cytochrome a_3 and the "invisible" copper) involved in the binding of CO and the reaction with molecular oxygen. In the purified enzymatically active cytochrome b-c_1 complex, there are also 4 one-electron redox components in equal stoichiometry - cytochromes b_{561}, b_{565}, and c_1 and the Rieske iron-sulfur protein. Ubiquinone is present in small amounts (0.3 moles/mole of cytochrome c_1). Titrations with oxidants and reductants (chemical or coulometric) establish that all of the electron acceptors and donors present are accounted for by known redox components. Experiments using submitochondrial particles confirm the results obtained using purified cytochrome b-c_1 complex and cytochrome c oxidase. The purified preparations of the oxidase and the b-c_1 complex represent redox components of the 2nd and 3rd phosphorylation sites of the respiratory chain respectively. The similarity of composition between these preparations indicates that the energy transduction mechanisms at the two sites are very similar.

THE TEMPERATURE DEPENDENCE OF THE REDOX POTENTIAL OF HORSE HEART CYTOCHROME C IN SODIUM CHLORIDE SOLUTIONS

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Abstract: The $E^{o'}$ values for the conversion of horse heart cytochrome c from the oxidized to the reduced form as a function of temperature have been measured in 0.10 M NaCl, 0.10 M sodium phosphate, pH 7.0 solutions in H_2O and D_2O. Measurements were made by a spectropotentiostatic method utilizing a thermostated optically transparent thin layer electrode (Heineman, Norris and Goelz, (1975) Analytical Chemistry, 47, 79). In H_2O, the decrease in the $E^{o'}$ value is linear with increasing temperature up to 42°C. Above this temperature, the decrease is again linear but with a much greater slope. In D_2O solutions, however, this biphasic behavior was not observed but instead a single line was obtained over the temperature range studied (25°C to 50°C). These results are interpreted in terms of the ability of NaCl to cause a destructuring of the bulk H_2O above 42°C but not in the more stable D_2O (Kreishman, Foss, Inoue and Leifer (1976) Biochemistry, 15, 5431). This decrease in water structure results in a shift in the equilibrium to the larger oxidized form as indicated by the decrease in $E^{o'}$. The involvement of specific chloride ion binding to the oxidized form will be discussed.

ELECTROCHEMICAL STUDIES OF CYTOCHROME C

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Abstract: Electrochemical studies have been carried out in an effort to 1) more fully develop and demonstrate the use of electrochemical methodology in kinetic studies of oxidation-reduction proteins and 2) to more completely elucidate the role of charge effects in the heme crevice region of cytochrome c upon the electron transfer mechanism. Two different approaches have been taken. In the first approach, an electrochemical characterization has been made of various iron EDTA and EDTA-like complexes. Chronamperometric methods were then used in obtaining the rates of electron transfer between native cytochrome c and the ferrous complexes In the second approach using the same electrochemical methods, the reduction of selected chemically modified cytochromes was studied. In one group of modified cytochromes c the lysines which surround the heme crevice were chemically modified but with no apparent change in conformation of the protein; in a second group internal modifications were made which change the shape of the heme crevice but not its charge. These studies indicate the importance of electrostatic interactions between the two redox couples upon the electron transfer mechanism.

ENERGETIC ANALYSIS OF CHEMICAL AND CHEMIOSMOTIC HYPOTHESES OF MITOCHONDRIAL AND CHLOROPLASTIC OXIDATIVE PHOSPHORYLATIONS

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Abstract: The choice between chemical and chemiosmotic hypotheses of oxidative phosphorylations stay ambiguous from experimental grounds.

The experimental arguments in favor of, and opposed to both concepts are reviewed.

- mere generalisation of other well understood conversion of redox energy to hydrolytic energy, but difficulty of proving the formation of any short life intermediate as it concerns chemical hypotheses

- evidence of the existence of proton transport effects but impossibility of proposing any properly said elementary process of conversion of gradient or field into condensed compound, as it concerns chemiosmotic hypotheses.

Consequently both hypotheses are analysed from energetic grounds taking into account relationships between energetic properties of redox couples belonging to the electron transfer chains and of ATP condensation and local parameters connected to H^+ and earth alcaline cations.

The importance of physiologically defined parameters, such as e/H^+ transfer ratio, regarding the choice between both theories is put into evidence.

THE KINETIC MECHANISM OF ACTION OF AN UNCOUPLER OF OXIDATIVE PHOSPHORYLATION

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Abstract: The chemiosmotic hypothesis predicts that the mechanism by which weak acids uncouple oxidative phosphorylation in mitochondria is identical to the mechanism by which they transport hydrogen ions across artificial bilayer membranes. We report here the first kinetic study of uncoupler-mediated hydrogen ion transport across bilayer membranes. We made electrical relaxation measurements on black lipid membranes exposed to the substituted benzimidazole 5,6-dichloro-2-trifluoromethylbenzimidazole. The simplest model consistent with out experimental data allowed us to deduce values for adsorption coefficients and rate constants. Our major conclusions are that the back diffusion of the neutral species is the rate limiting step for the steady state transport of hydrogen ions, that both the neutral and charged forms of the uncoupler adsorb strongly to the interfaces and that the reactions at the membrane solution interfaces occur sufficiently rapidly for equilibrium to be maintained. Independent measurements of the adsorption coefficients of both the neutral and anionic forms of the weak acid and also of the permeability of the membrane to the neutral form agreed well with the values deduced from the kinetic study. We also obtained steady state conductance data for a variety of uncouplers which allow us to reconsider the correlation between the effectiveness of weak acids in transporting hydrogen ions across artificial membranes and in uncoupling mitochondria. Our data support the chemiosmotic hypothesis.

This work was supported by NSF grant PCM 04363.

ELECTROCHEMISTRY OF VITAMIN B 12. SPECTROELECTROCHEMISTRY OF REDOX AND ACID-BASE EQUILIBRIA IN THE B 12a/ 12r SYSTEM

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Abstract: Analysis of the B 12a/B 12r system on a gold or platinum electrode by cyclic voltammetry shows that reversibility is far to be reached even at the lowest usable sweep rates. A spectroelectrochemical method using a platinum electrode with long electrolysis durations is therefore employed to determine the standard potential as a function of pH in the range -1 to 11. Joining these results to those previously obtained with the B 12r/B 12s couple leads to a general picture of the stability ranges of the three oxidation states of aquocobalamin as a function of potential and pH. Implications on B 12r disproportionation and on the expectable redox behavior of aquocobinamide and cobyric acid are discussed.

PHOTOELECTROCHEMISTRY OF PIGMENTED LIPID MEMBRANES

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Abstract: The lipid bilayer as an ubiquitous component of the structure of biological membranes seems firmly established on the basis of electron microscopy, chemical analysis, and physical investigations of natural organelles and their membranes. Closely associated with these structure studies are the functions of biological membranes, the most important of which is energy transduction. Insofar as the transduction of light into electrical energy and/or chemical free energy is concerned, two kinds of photoactive membrane systems have been evolved in nature, namely, the thylakoid membrane of chloroplasts and the sac membrane of photoreceptors. In the former the principal function of the thylakoid membrane is the conversion of light into chemical free energy via the separation of charges, whereas in the visual sac membrane the task is that of a light-activated sensor detecting the presence of photons thereby in some obscure fashion which alters the electrical polarization across the membrane. The molecular construct of either photoactive membrane systems is not yet known in detail, but in each of these light transducers the key element is believed to be a pigmented ultrathin lipid membrane separating two aqueous phases. Ideally, a great deal of photoelectrochemistry of these pigmented lipid membrane systems can be learned from electrical measurements by placing electrodes across such membranes, much as has been done for the nerve membrane of squid axon. Unfortunately, such an approach is not yet feasible at present for most photoactive membranes, particularly those of subcellular organelles such as aforementioned thylakoids or photoreceptor sacs, owing to their minute size. Artificial pigmented bilayer lipid membranes (BLM), therefore, appear to offer the most viable system in which to investigate energy conversion processes and light-initiated redox reactions.

In this paper I shall be concerned with light mediated phenomena in membranes of photosynthesis and vision and their in vitro model bilayer lipid membranes (of both planar and spherical configuration) containing appropriate photoactive compounds. Chloroplast extract BLM, carotenoid BLM and Halobacterium halobium (bacteriorhodopsin) BLM are used as examples in the presentation. Particular stress is placed on those molecular mechanisms of photoelectrochemical energy transduction in these pigmented lipid membranes which are relevant for the elucidation of photosynthetic and visual processes. It will be apparent that the experiments with photoelectric lipid membranes are still at an early stage of development and wide open for the application and fusion of ideas from electrochemistry, photochemistry, and membrane biology.

MECHANISM OF GENERATION OF THE EARLY RECEPTOR POTENTIAL REVISITED

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Abstract: The early receptor potential (ERP), which was discovered by Brown and Murakami in 1964, is generally regarded as a displacement current caused by a light-induced change of electric dipole moment of the oriented visual pigment molecules in photoreceptor membranes (Oriented Dipole mechanism or OD mechanism). It has been considered that its amplitude is too small to play any significant role in visual transduction process. In the light of recent progress in the studies of photoelectric effects in artificial pigment-containing lipid bilayers, these conclusions may be premature.

A transient photoelectric signal can be elicited by a brief light pulse from a model lipid bilayer system which contains lipid-soluble magnesium porphyrins and separates an aqueous redox gradient of potassium ferricyanide and ferrocyanide solutions. It was found that this transient response possesses all the major characteristics of the ERP. In particular, it is also a displacement current. Therefore, an alternative interfacial charge transfer (ICT) mechanism can generate an ERP-like photosignal just as well.

Theoretical analysis of the ICT model in terms of Gouy-Chapman diffuse double layer theory indicates that the displacement current is a consequence of the ultra-thinness of membranes and a discrepancy of ionic cloud relaxation between the aqueous phase (extremely fast) and the membrane phase (extremely slow). Furthermore, the displacement current is not a manifestation of the membrane capacitance as is commonly believed. It is a manifestation of a physically distinct novel chemical capacitance. Similar analysis of the OD model leads to a chemical capacitance of different physical construction, which is, in principle, distinguishable from the one due to an ICT mechanism.

The only known charge transfer reaction in visual pigment photochemistry is the metarhodopsin I to metarhodopsin II transition. This reaction has been correlated with the time course of the R_2 component of the ERP. Thus, the R_2 component may be generated by an interfacial proton transfer mechanism.

Model system studies indicate that it is far more sensitive to measure transient photoelectric responses by short circuit methods than by open circuit methods, and that intrinsic photoelectric relaxation tends to be obscured by the membrane RC relaxation under open circuit condition. We suggest that the tunable voltage clamp method introduced by Hong and Mauzerall (J. Electrochem. Soc. 123, 1317 (1976)) may be applied to the study of the ERP mechanism.

Model system studies also suggest that a small ERP (as observed by open circuit methods) may have an intense local electric field effect near the membrane-water interface. The possible physiological significance of ERP will be discussed.

LINEAR FREE ENERGY RELATIONSHIP BETWEEN LIGHT AND ELECTROCHEMICAL REACTIONS OF BASES IN NUCLEIC ACIDS

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Abstract: Excited sensitizers adsorbed at nucleic acid - or protein - surfaces may cause photooxidation of certain bases or amino acids leading to photodynamic effects in living beings. Working with flash photolysis we registered some intermediates of the system thiopyronine (TP)and bases. We found that the oxidized radical of the sensitizer $\overset{\bullet}{TP}^+$ changes quanine G to $\overset{\bullet}{G}^+$ which starts fissions and strand break simultaneously. Further photodynamic experiments with some other dyes and the normal nucleic acid bases as well as their aza-analogues and minor bases shows clearly that the difference between oxidation potentials of sensitizers $S \rightleftharpoons S^+ + e^-$ and of bases $B \rightleftharpoons B^+ + e^-$ measured at graphite electrodes may be of great importance for the rate of photodynamic reaction pathway. From such a kind of linear free energy relationship we also discovered stronger sensitizers looking for their electrochemical oxidation potentials. By the way, we succeeded in finding a stable sensitizer for chemical oxidation of water suitable for light conversion in photogalvanic cells.

Photodynamic experiments were done with numerous dye-stuffs as sensitizers and the normal nucleic acid bases as well as their aza-analogues and minor bases as substrates.

Degradation of substrate molecules was performed mainly in using sensitizer with oxidation potentials above that from the corresponding substrate. This finding supports the idea that oxidized dye radicals are the photodynamically active agents.

ELECTROCHEMICAL STUDY ON TRANS- AND CIS-RETINAL AND BACTERIORHODOPSIN IN ETHANOL - AQUEOUS MEDIA

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Abstract: Bacteriorhodopsin is a good example in biology of an ion pump and much interest exists in its characterization because the question of how hydrogen and electron carriers function as ionophores is one of the main problem of bioenergetics. There are some evidence that, the energy transfer-system in bacteriorhodopsin might be connected with changes in conjugated π-electron system of retinal (1).

A study has been made of the polarographic behaviour and the mechanism of electroreduction of all-trans and cis-retinal in 40% ethanol - aqueous buffered system. Both compounds involve the same redox pattern of two successive one-electron additions. The initial step involves the simultaneous addition of an electron and of a proton to form a free radical, which dimerizes. The electroreduction products of these two reduction steps of all trans-retinal have been identified by means of UV, IR, and mass spectroscopy.

Bacteriorhodopsin isolated from halobacteria (2) has been investigated in buffered aqueous system by d.c. polarography. Bacteriorhodopsine gives one polarographic wave, which pH dependence of $E_{1/2}$ is similar as it was found for the first reduction step of trans- and cis-retinal.

(1) D. Oesterhelt, M. Meentzen and L. Schumann, Eur. J. Biochem. 40, 453-463 (1973).
(2) P.J. Bauer, N.A. Dentcher, Biophys. Struct. Mechanism. 2, 72-92 (1970).

CORRELATION OF TRANSITION ELECTROPOTENTIALS AND STEM DIAMETER CHANGES IN LYCOPERSICON ESCULENTUM

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Abstract: Simultaneous measurements of transition electropotential (potential pulses occurring at lights on and lights off) and stem diameter were made with lycopersicon esculentum under relatively normal diurnal light cycles over a period of weeks using penetrating palladium probes. The resulting electrophytogram (potential plotted against time) indicates juvenile tissue has a 75% greater transition electropotential magnitude than mature tissue on the same plant. The timing of the transition electropotential does not correlate with the timing of the concomitant stem diameter change. The magnitude of the dusk transition electropotential has a highly significant correlation ($P > 0.1\%$) with the long term changes in stem diameter.

PROTEOLYTIC STUDIES OF CHAIN CLEAVAGE AND PROTON PUMP ACTIVITY OF BACTERIORHODOPSIN IN PURPLE MEMBRANES.

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Abstract: The protein of the purple membrane of Halobacterium halobium contains retinal covalently bound to a lysine residue by means of a protonated Schiff base. The function of this protein is to act as a light-driven proton pump producing a trans-membrane proton gradient which is coupled to ATP synthesis. Some of the molecular details of the phototransducing mechanism have been investigated by enzymatic degradation of purple membrane fragments, using trypsin, papain and pronase, both in "dark" and "light"conditions. The extents of chromophore loss and protein cleavage have been determined and the protein chain fragments have been characterized by SDS-polyacrylamide gel electrophoresis. The extent and the kinetics of the light-induced proton transfer in reconstituted proteoliposomes incorporating the enzymatically modified protein have been studied.

Trypsin cleaves off a small fragment of the protein chain, leaving chromophore and proton pump essentially unchanged. For papain, a small loss in both protein and chromophore is sufficient for completely abolishing the slow phase of the proton pump induced pH change. Pronase treatment results in a large, and light-dependent, loss of chromophore, however the kinetics of the pH change is identical with that of the papain treated protein. It appears that the enzyme sensitive site that is required for the function of the proton pump is located in a part of the bacteriorhodopsin molecule different from that of the chromophore site.

EXCITABLE MEMBRANES: IONS, CHANNELS, GATES AND SPACES

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Abstract: The concept of discrete ion conducting channels across the membranes of excitable cells will be considered. Methods for obtaining counts of the density of these channels per unit surface area of membrane will be discussed as well as methods for determining single channel conductances. Analogies will be made to the channels formed by macrocyclic polypeptide molecules in lipid bilayers. The voltage dependent gating of ionic currents in excitable cells is considered in light of voltage clamp experiments and mathematical models of gating kinetics and gating currents. Ion interactions within membrane channels will be discussed in terms of ion competition, saturation, and inhibition. External diffusion barriers and spaces in which ion accumulation occurs will be considered as modifiers of excitable membrane function. Appropriate alterations will be developed in the Hodgkin-Huxley nerve equations to account for the role of these barriers and spaces in modulating neuronal function.

MOLECULAR EVENTS AND ENERGETICAL CHANGES DURING THE ACTION POTENTIAL

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Abstract: A novel interpretation of the existing data concerning the energetical changes associated with nerve impulse propagation is proposed. The initial heat of activity expresses the extra-dissipation of free internal energy due to specific processes occurring in the nerve fiber during the AP. In the apparent order of their appearance these processes are:

a) structural changes in the membrane as a result of the biochemical events in the membrane proteins induced by the changes in the electric field;

b) redistribution of electric charges in the membrane (capacitive current)

c) ionic flow through specific ionophores

Owing to the authoritative domination of the ionic theory only processes (b) and (c) were considered, while the energetics of structural changes occurring in the membrane itself was practically neglected. The classification of the processes occurring in the active axon as presented above could have a structural relation if one assumes that:
process a) is related to specific proteins in the membrane;
process b) is mainly (but not exclusively) dependent on the membrane lipids;
process c) expresses the changes in the ionic compartment and the frictional interaction of the ions with the membrane.

When taking into account the known data concerning the electrical properties, the ion fluxes, and the number of ionophores per surface area in the squid giant axon, the crab nerve and the rabbit vagus nerve, a very good quantitative agreement between the computed heat changes and the microcalorimetric measurements is found. The most distinctive feature of our treatment is the proposal that the closing of the ionic channels explains together with the recharging of the membrane capacitor, the heat resorption. As so often stated the ionophores cannot use the thermal energy previously delivered. Two biochemical systems that could provide the adequate amount of energy will be discussed in relation to the presentation of the thermodynamical data.

ELECTRIC FIELD INDUCED CONFORMATIONAL CHANGES IN EXCITABLE NERVE MEMBRANE MAY BE STUDIED BY CHANGES IN THE INTRINSIC FLUORESCENCE OF MEMBRANE PROTEINS.

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Abstract: Electric field dependent changes in the gating of channels for sodium and potassium may be the molecular mechanism for nerve excitation. While the use of extrinsic fluorescent probes and the study of the optical properties of nerve membrane during electrical excitation may permit one to monitor the propagation of the nerve impulse the signal contains little information related to the molecular structural changes that are involved. We have studied the changes in the intrinsic fluorescence of the aromatic amino acids groups of nerve proteins which undergo a change in their intrinsic fluorescence during the propagation of the nerve impulse. We studied changes in the intrinsic fluorescence of garfish olfactory nerve fibers (Lepisosteus osseus) in relation to single action potential. Changes in the fluorescence intensity, polarization, lifetime and quenching together with the use of specific nerve blocking agents can be used to localize the molecular structure that is involved in the electric field dependent current gating mechanisms.

IONIC CURRENT AND SURFACE POTENTIAL OF AXON MEMBRANES

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Abstract: Ionic currents across squid axon membranes under various clamped voltage conditions are examined with variation of the extracellular Ca^{2+} concentration.

It was found that the maximum Na^{+} current under voltage clamp was decreased with increase in the extracellular Ca^{2+} concentrations, whereas the maximum K^{+} current was unchanged.

The well known shift of the clamped voltage-ionic conductance current relation to the voltage axis and the changes in maximum ionic currents with respect to Ca^{2+} concentrations in the bulk aqueous phase are analized in terms of surface potential and surface ion concentrations of the axon membrane.

A new interpretation of the relation between ion equilibrium potentials (or ion reversal potential) and ionic concentrations is proposed, and an experimental examination of this proposal has been performed with squid axon membranes.

ION FLUXES, CONCENTRATION DISTRIBUTION AND ELECTRIC POTENTIALS OF SQUID AXON MEMBRANE SYSTEMS

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Abstract: On the basis of previously obtained equations, and values of partial frictional coefficients extracted from experimental results, the state of affairs in stationary state of the squid (loligo Forbesii) axon membrane system is discussed. Comparison of theoretically predicted results with experimental observation regarding concentration distributions and electric potentials are presented.

Under the assumption of lack of water transport, the concentration profiles in interfacial regions and membrane phase are analyzed. Some considerations of unstirred diffuse layers are presented.

MOLECULAR MECHANISM OF ANAESTHESIA

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Abstract: The presence within bimolecular lipid membranes of substructural layers with different dielectric and/or conductance properties manifest a dispersion with frequency of the capacitance and conductance of such membranes at very low frequencies (0.001 - 1000 Hz).

Recent techniques developed to measure this dispersion were used to determine the effects of local anaesthetics on the substructure of lecithin/cholesterol bilayer membranes.

It was found that the local anaesthetics procaine and benzocaine greatly reduced the hydrocarbon region thickness of these membranes. Benzyl alcohol, on the other hand, increased the thickness of this region. The mechanism by which this occurs can be readily seen from considerations of molecular packing.

On the basis of the fluid-mosaic model of the nerve membrane such considerations show that the excitation conduction channel will be stressed by the action of the anaesthetic on the bilayer structure in which this conduction channel is embedded.

RELATIONS BETWEEN THE ELECTRETS AND THE NERVE CONDUCTION

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Abstract: In this study, an electret theory based on a continuum mixture model, and, the relations between the electrets and the nerve conduction, are given.

An electret is a piece of material which is permanently electrically polarized and it is usually prepared in the form of flat plate, having a permanent polarization in the direction of the thickness. Electrets are made from the following materials. These materials are; certain waves, plastics, polymers and some poor electrical conductive materials.

In recent years the electrets have found lots of places to be used, such as electrical transducers, biomedical polymers and so on. The biomedical polymers, have been used in medicine for the replacement of the segments of large blood vessels and heart values where their artificially obtained uniform negative surface charges, are the main factor for antithrombogenic character. A theory which is given for electrets can be easily applied to the nerve conduction. An electret during the fabrication by thermoelectrical means reaches to a state where the charged groups in the material are freed by heating and mobilized by an electrical field. Thus causing a surface charge and permanent polarization which can be locked in to the material by cooling.

The state of mobil ions in electrets are similar to the excitable membranes. These ions cause conduction in the excitable membranes and are controlled either by the negative surface charge of the membranes or the electrical charged groups in the pores of the membrane.

Therefore the surface charge of the excitable membranes must be taken into account in addition to the other physical factors in the electrets in order to find out the distribution of the ions in the resting potential state.

DISSOLUTION OF Pt STIMULATION ELECTRODES IN SALINE MEDIA

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Abstract: In vitro studies have shown that appreciable dissolution of Pt electrodes can occur during the application of electrical pulses analogous to those used for neural stimulation. Square wave current pulses of zero net charge ("balanced biphasic pulses") and of 0.1 to 10 mS duration were applied at 50 Hz to Pt bead electrodes in saline solution. Dissolution was monitored by periodic analysis of the solution for Pt. Dissolution rates, expressed in terms of faradaic charge, ranged from $< 10^{-4}$% to 10^{-1}% of the anodic charge per pulse. Law rates were favored by small charge densities and by the presence of protein in solution. Other important factors include the electrode surface preparation, the pulse polarity (anodic phase first being worse than cathodic phase first) and the concentration of Pt already in solution. There is relatively little effect of current density (up to 1 A cm^{-2}), temperature (up to 37°C), or pH buffering.

BIOELECTROCHEMICAL STUDIES OF THE IMPLANTABLE BONE STIMULATION ELECTRODE

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Abstract: The mechanism of electrical bone stimulation, although having been applied to 1000 human patients during the last six years, remains a mystery. Several metal electrodes and different current waveforms have been reported to be effective, although continouous DC current cathodes have been used in the majority of cases. In order to discover the mechanism, we are studying the effects of such electrodes on relevant cell preparations, both in vivo and in vitro and trying to correlate these effects with the electrochemical behavior of the electrodes in the biological milieu.

Studies of the marrow cell population distribution surrounding Ag, Au, Ti, Pt, graphite and stainless steel electrodes in vivo have shown that cell-specific and metal-specific changes occur in a short time. In general, cathodes caused a decrease in the differential lymphocyte population and increases in eosinophils. The latter was particularly striking for Pt. Stainless steel, a metal in common use as a bone electrode, caused significant cell lysis.

In vitro cultures of fibroblasts, exposed to these electrodes, have suffered some loss of viability around Ag and Pt electrodes. Ag anodes produced a curious morphological change in these cells, which is, at least in part, reversible.

In vivo and in vitro electrode measurements are being made to characterize the possible reactions that may occur and correlate these with the biological effects, particularly the osteogenic response. Rapid impedance measurements as well as steady-state V-I characteristics show thus far that electrode changes of possible biological importance occur with time.

Several classes of mechanisms which could be responsible for stimulating osteogenesis involve: (1) direct cellular processes (field or ion effects on cell membranes), (2) specific electrochemical products, (3) non-specific inflammation. The incomplete evidence at the present time suggests that each of the three types is involved in electrical osteogenesis, depending on the situation. The problem is to separate them experimentally.

BIOELECTROCHEMICAL SYSTEMS' MODELS, ELECTROMAGNETIC INTERACTIONS AND NOISE

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Abstract: A general approach to the modelling of bioelectrochemical processes is discussed, which relies on multi-compartment models. This approach can be applied to different types of biosystems, e.g., blood cells, ion flows through membranes, etc.

Once compartment populations have been specified, continuity relationships give a biosystem's state equations.

The basic idea is that each net input flux ϕ_i entering the various compartments can always be split into two unidirectional microscopic fluxes ϕ_i' and ϕ_i'', such that $\phi_i=\phi_i'-\phi_i''$, where ϕ_i' is a true input (I) flux or a production (P) rate, and ϕ_i'' is a true output (O) flux or a disappearance (D) rate.

In doing so, it is always possible to define a zero-rate equilibrium condition $\phi_i'=\phi_i''$ which allows us to apply Onsager's reciprocity conditions to model equations correctly, in order to test their physical reliability.

A general approach, based on mass-action law statistics, is proposed which enables us to compute unidirectional nonlinear fluxes as functions of compartment populations, when actual bioelectrochemical forces are not known in detail.

Possible interactions of the populations that are electromagnetically active with electromagnetic fields are discussed and modelled, reviewing the overall energetic requirements of the Poynting theorem.

Finally, the noise associated with ϕ_i^k (k=I,O,P,D,) is considered. Each microscopic unidirectional flux is viewed as a Poisson process. The 'individuals' of each population transfer from a compartment to another at random times, so that the noiseless value $\phi_i^k(t)$ gives the average occurrence rate of the corresponding Poisson process at t during any transient. We can show that the related autocorrelation noise function $R^k(t_1,t_2)$ can be evaluated from the noiseless flux: $R^k(t_1,t_2)=\phi_i^k(t_1)\ \delta(t_2-t_1)$, $\delta(t_2-t_1)$ being the Dirac delta function.

Computation of the noise of each compartment population becomes now straightforward. Therefore, it becomes apparent that a quantitative amount of information on the fluxes' values can be obtained from noise measurements.

Recent results relevant to the white cell system of human beings are presented.

AN INTERFACIAL ELECTROCHEMICAL APPROACH TO THE CONTROL OF CELLULAR BEHAVIOR

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Abstract: The concept of electrochemical information transfer in vivo was proposed by the author several years ago in the context of studies involving the control of bone growth and repair. This study invokes the proposal that dynamic interfacial electrochemical phenomena can be basic biological steps in the (control) of cell function. Of particular importance in this approach is the potential dependent step of specific adsorption (binding) which appears to be involved in such cellular phenomena as regulatory enzyme activity, protein synthesis, differentiation, malignant transformations, cell-cell and cell-tissue interactions. A detailed interfacial membrane model has been developed and utilized to predict that highly specific charge injection can affect surface interactions in a manner to modify cellular behavior. Earlier studies suggested that Ca^{2+} ion is a predominant entity in the response to electrical perturbation. Utilization of charge injection with parameters satisfying an interfacial electrochemical approach, the rate of Ca^{2+} uptake in embryonic chick bone explants has been studied. The results show that the excitation of a small ($\simeq$ 300 μsec) or large ($\simeq$ 5 msec) time constant can elicit significant differences in the rate of Ca^{2+} uptake even when the total injected charge is identical. These results are discussed in the light of the known effects of Ca^{2+} upon enzyme activity, contractile proteins, cell division and particularly its apparent potential dependent action upon transmembrane ion transport.

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POSSIBLE MECHANISMS OF WEAK EM FIELD COUPLING IN BRAIN TISSUE

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Abstract: We have observed that extremely weak oscillatory electric fields influence calcium exchange in isolated cerebral tissue. Chick forebrains pre-incubated with radioactively labelled $^{45}Ca^{++}$ were exposed to a radio frequency field (147 MHz, 0-8 mW/cm^2), amplitude modulated at brain wave frequencies (1 to 35 Hz). Significant increases in the efflux of labelled calcium (up to 15%) were seen to be dependent on the modulation frequency of the carrier wave. Frequencies between 6 and 20 Hz produced significant results and similar frequencies were effective in experiments where chick and cat cerebral tissues were exposed to sinusoidal extremely low frequency fields (1 to 75 Hz). $^{45}Ca^{++}$ efflux decreased after exposure to field frequencies of 6 and 16 Hz but other frequencies (1, 32 and 75 Hz) did not significantly effect calcium exchange. Moreover, the effect was seen only for field amplitudes greater than 5 V/m but less than 100 V/m. A similar upper threshold for field intensity occurred with 450 MHz fields amplitude modulated at 16 Hz: $^{45}Ca^{++}$ efflux increased after exposure to field powers from 0.375 to 1.0 mW/cm^2, but was not altered by higher field intensities (2.0 and 5.0 mW/cm^2).

The rhythmic oscillation of the imposed fields could be transduced by the neuronal membrane. In current membrane models the semi-fluid mosaic of proteins embedded in the phospholipid bilayer is associated with an outer layer of interlaced polar glycoproteins that provide negative binding sites for extracellular cations as an aspect of their structural asymmetry. The observed increase and decrease in $^{45}Ca^{++}$ efflux could reflect a change in the binding of calcium to oxyanions which can vary in strength over several orders of magnitude. Because the effect occurs only for frequencies in a narrow window coinciding with the EEG range these data suggest an extracellular regulatory mechanism functioning at extremely low frequencies.

Current studies on isolated cells of the Aplysia abdominal ganglion examine the response of functioning neurons exposed to weak, extremely low frequency fields. The Aplysia preparation may provide detailed information on characteristics of the transduction of such fields by the neuronal membrane.

Studies of simultaneous efflux of $^{45}Ca^{+}$, ^{3}H-GABA and ^{3}H-β-alanine from isolated chick forebrains exposed to ELF fields (18 Hz 1.20 V/m) have not shown significant alterations in amino acid efflux, although $^{45}Ca^{2+}$ efflux followed the pattern described above.

BRAIN DOPAMINERGIC NEURONS: IN VIVO ELECTROCHEMICAL INFORMATION CONCERNING STORAGE, METABOLISM AND RELEASE PROCESSES

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Abstract: The potential usefulness of modern voltammetric techniques for investigations of brain neuronal functioning has gained increased interest in recent years. The advantages of the approach are basic: it enables one to measure concentrations of species directly, and it does this simply, quickly and in the presence of numerous other components in a complex system, negating the need of first isolating the molecular species of interest. Interfering species can be compensated for in most cases. Voltammetric microelectrodes have the additional advantage of making measurements possible for the first time in vivo under conditions that approach normalcy and, in addition, in an instantaneous, localized, non-destructive, phase-selective and continuous manner.

In this paper, cyclic voltammetry, differential pulse voltammetry and differential double pulse voltammetry, utilized with chemically modified platinum or carbon electrodes, are evaluated for their ability to detect the chemical neurotransmitter dopamine and its major metabolite homovanillic acid in mammalian brain. Results are presented demonstrating the applicability of the methods developed to studies of transmitter storage, metabolism and release processes following selective pharmacological manipulation of neuronal pathways. Certain problems inherent to such measurements in brain tissue are discussed.

LANTHANUM INHIBITION OF ELECTRICALLY INDUCED DEDIFFERENTIATION IN FROG ERYTHROCYTES

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Abstract: Erythrocytes from adult Rana pipiens pipiens were diluted 1/400 in isotonic saline and isotonic Ca-Mg free PBS. When subjected to pulsed D.C. fields @ 50Hz (10 msec on/off pulse) and 100 mv (50 mv/cm) in a modified tissue culture dish, the cells dedifferentiated in all media in the same manner as commonly seen by Becker (1967) and Pilla (1974). Addition of $LaCl_3$ at 10^{-4}M or above prevented the dedifferentiation. Blockage was partial at 10^{-5}M and absent at 10^{-6}M. The dedifferentiation could also be produced by adding 10^{-6}M calcium Ionophore A23187. Ionophore-induced effects could be blocked with $LaCl_3$, and released again with electrical stimulation. We conclude that transmembrane exchange of Ca^{++} is a critical step in electrically induced dedifferentiation of these cells.

REGENERATION IN DENERVATED LIMBS OF SALAMANDERS AFTER INDUCTION BY APPLIED DIRECT CURRENTS

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Abstract: Normal limb regeneration has been obtained in denervated limbs of salamanders by applied direct currents. The currents used were of the magnitude, polarity and length observed in regenerating limb stumps with nerves. Regeneration was observed to begin after 12 hours of treatment when the tip of the stump was made negative. It never began when the tip was made positive. The first signs of regeneration were loss of tissue structure, conversion of tissues to cells with subsequent division of the cells.

COMPARISON OF THE EFFECTS OF MINUTE LEVELS OF DIRECT ELECTRIC CURRENT AND NERVE GROWTH FACTOR ON EMBRYONIC SENSORY GANGLIA IN VITRO

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Abstract: Minute levels of electric current have been shown to increase the growth of nerve fibers from chick embryonic sensory ganglia grown in vitro (Sisken and Smith, 1975). This stimulation by direct current resulted in fibers which were more numerous, longer and more highly branched, and which closely resembled those grown by sensory ganglia treated with nerve growth factor.

These investigations have now been extended to include both morphological (SEM) and biochemical (isotope incorporation) studies to more clearly define the d.c. effects and to establish any commonality they may possess to those published for nerve growth factor. The scanning electron microscopy (SEM) affords a three dimensional view of the complex arrangement of nerve fibers, glial cells that are apposed to the fibers, and the fibroblast mat upon which the fibers and glial elements grow. In order of complexity, cultures treated with direct current have a more profuse outgrowth than controls, but do not show as luxuriant an outgrowth as cultures treated with nerve growth factor.

In an attempt to ascertain the nature of the effects of current or nerve growth factor on a molecular basis that might be reflected in an increased growth pattern, we have followed the incorporation of isotopically-labeled compounds into macromolecules. Incorporation of leucine-H3 into proteins was determined by liquid scintillation for control cultures, cultures treated with 10-20 nA direct current, and cultures treated with nerve growth factor (10 BU/ml). After 20 hours of incubation in an isotope-containing medium the following results were obtained: control ganglia (820 counts/min/ganglia), direct current treated (1290 counts/min/ganglia) and nerve growth factor treated (1650 counts/min/ganglia).

The conclusion we have drawn thus far is that anabolic stimulation by direct current resembles that produced by nerve growth factor both morphologically and biochemically but to a lesser degree. The mechanisms by which either of these treatments exert their effect is still unknown.

CALCIUM - MEDIATED MORPHOGENETIC MOVEMENTS DURING NEURULATION

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Abstract: As amphibian embryos form their nervous system they release Ca^{2+} to the spring water medium. Papaverine, which interferes with Ca^{2+} flux, inhibits the movements enclosing the neural tube, while ionophore A23187 and E6TA increase Ca^{2+} flux causing a rapid cellular constriction leading to neural fold formation.

These effects were followed in the transmission electron microscope where both papaverine and ionophore were seen to mediate dramatic effects at the level of the microfilaments. Papaverine induces disruption of these organelles and ionophore promotes rapid reconstitution and constriction.

Ca^{2+} flux was monitored as a concomitant of these drug treatments with flame emission atomic absorption spectrometry. The morphological changes in the embryonic surface were studied with scanning electron microscopy and x-ray probe microanalysis.

ELECTRICAL AUGMENTATION OF BONE GROWTH AND REPAIR

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Abstract: During the past 20 years, it has become possible to stimulate bone growth and repair by electrical means, both in animals and in humans. At the present time, fresh fractures and fractures, which have failed to heal, are treated electrically with considerable success in a number of centers both in this country and elsewhere. Two different approaches have been employed to achieve these results. The first involves a surgically invasive technique to implant current - delivering electrodes in bone. This method which involves passage of 50 to 700μ A/cm^2, produces a number of electro-chemical sequellas which may trigger bone formation as a non-specific result of electrochemical (faradaic) irritation. The second method involves production of current in tissues by inductive-coupling of time-varying electromagnetic fields. Current levels in this latter system are two to three orders of magnitude less than in the electrodes systems and evoke a response in the absence of significant alterations in temperature and without faradaic reactions in the environment of cells. Furthermore, the pulse characteristics required to stimulate bone growth or repair, are highly specific in terms of amplitude, width, energy distribution, and repetition rate. Two different, clinically effective pulses have been shown to have a high degree of specificity of effects on cell function. One increases calcium release by cells in tissue culture and the other increases protein synthesis. With surgically-non-invasive, time-varying electromagnetic fields, more than 100 patients with surgically-resistant non-unions of fractures or pseudarthroses have been treated during the past four years with an 85% success rate. It appears that this approach holds great promise for many diseases and disorders, not only of the musculoskeletal system, but of other systems as well.

CURRENT STATUS ON THE ELECTRICAL STIMULATION OF BONE GROWTH

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Abstract: Over the past five years, interest has increased in the clinical use of electrical stimulation to enhance bone growth. At this time a variety of modalities are in use, all producing bone growth stimulation. These include, low intensity D.C., high intensity D.C., A.C. and pulsed magnetic fields. It would appear improbable that all of these different modalities are operating through the same mechanism. A suggested explanation will be offered along with a short review of the techniques employed in our laboratory and the clinical results thus far.

COMPARISON OF IN-VITRO AND IN-VIVO PASSIVATION STUDIES ON IMPLANTABLE ANODES

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Abstract: In the corrosion studies on the sacrificial anodes of implantable hybrid cells used to supply energy to pacemakers and biotelemeters, the in-vivo anodic polarization curves for aluminum and zinc demonstrated an increased passivation for loaded electrodes over in-vitro measurements. Organic compounds which adsorb on metal surfaces have been known to suppress metal dissolution on both anodic and cathodic processes. Cystine has been shown previously by Svare, et al. to reduce the rate of dissolution of copper in human blood. Anodic and cathodic polarization measurements on aluminum anodes in amino acids, bovine serum albumin, and mucopolysaccharides which are being made in order to better understand the increased passivation of in-vivo implantations will be reported. The A.C. electrode impedance will be correlated with the above polarization measurements in the various media.

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